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Individual Differences in Plasma Catecholamine and Corticosterone Stress Responses of Wild-Type Rats: Relationship With Aggression

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SGOIFO, A., S. F. de BOER, J. HALLER, J. M. KOOLHAAS. *Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression.* PHYSIOL BEHAV **60**(6) 1403–1407, 1996.—Plasma noradrenaline (NA), adrenaline (A), and corticosterone (CS) responses to social and nonsocial stressors were studied in male members of a strain of wild-type rats, widely differing in their level of aggression. The aggressiveness was preliminarily established by measuring the latency time to attack (ALT) a male intruder in a standard resident-intruder test. Animals were then provided with a jugular vein cannula for blood sampling during stress exposure. Implanted rats were randomly assigned to 3 experimental treatments: social stress (defeat experience, SD), nonsocial stress (presentation of a shock-prod, SP) and control (animals undisturbed in their home cages, CTR). A significant correlation was found between ALT and the amount of time spent in burying the probe in SP rats: the more aggressive the animal, the higher the rate of burying behavior. SD induced a much stronger effect on plasma NA, A, and CS concentrations than SP. A significant negative correlation was found between ALT scores and values of the area under the response time curve for NA and A, in both SD and SP situations: the more aggressive the animal, the higher the catecholaminergic reactivity to the stressors. On the contrary, no evidence of a correlation between aggressiveness and plasma corticosterone responses was found, neither in SD nor in SP rats. These findings in an unselected strain of wild-type rats confirmed that an aggressive/active coping strategy is associated with a high sympathetic-adrenomedullary activation and support the concept of individual differentiation in coping styles as a coherent set of behavioral and neuroendocrine characteristics. *Copyright © 1996 Elsevier Science Inc.*

Stress Coping strategies Plasma catecholamines Plasma corticosterone Aggression

EXTENSIVE research shows that conspecific animals can differ in their behavioral and physiological reaction patterns to stressors. This differentiation in stress responsiveness is considered to be an important factor in the etiology of stress pathology. An experimental animal approach to stress and adaptation should consider stress-related pathology as a function of such an individual differentiation (2,17,30).

An analysis of the individual variation in behavioral, cardiovascular, and neuroendocrine reactivity in a wide variety of social and nonsocial challenging situations and in a number of species has led to the idea that at least two broad categories of coping styles exist (1,10,18,22). These are: an active coping strategy, whereby an animal displays high levels of motor activity in attempting to deal with or escape from an external threat (Cannon's fight/flight response), and a passive one, characterized by immobility and freezing upon aversive stimulation (conservation/withdrawal response) (7,11,24).

It has been claimed that the accompanying physiological and endocrine characteristics of these two behavioral coping strategies differ as well, the passive mode of response being attended by a high parasympathetic and adrenocortical reactivity whereas the active one is accompanied by a preferential sympathetic-adrenomedullary activation (3). Excessive or sustained recruitment of the sympathetic-adrenomedullary system in actively coping animals is generally considered to be an important factor in the development of hypertension and atherosclerosis (14,23). On the other hand, the excessive or sustained parasympathetic reactivity and/or activation of the adrenocortical axis in passively coping animals may render them more vulnerable to bradyarrhythmias and immunological disturbances (4).

However, the relationships between coping behavior and autonomic/endocrine physiology are primarily based upon correlations between independent measurements comparing commercially available strains or selected lines of rodents (16,26,31). The generality of the link between individual behavioral coping style and the associated differentiation in autonomic/endocrine physiology has hardly been investigated within one stock of rats. Therefore, the purpose of the present study was to examine

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plasma catecholamine (index of sympathetic-adrenomedullary activity) and corticosterone (indicator of adrenocortical functioning) responses to acute social (defeat) and nonsocial (shock prod presentation) stressing events, in individual members of a strain of wild rats that is supposed to differ widely in level of aggressive behavior.

METHOD

All procedures in this study were approved by the Committee on Animal Bioethics of the University of Groningen, The Netherlands.

Animals and Housing

Forty-two conventional male wild-type rats (*Rattus norvegicus*) originally derived from the Agricultural University of Wageningen and bred in the Department of Animal Physiology, University of Groningen (The Netherlands) were used. The animals were housed in unisexual groups of 5 individuals from weaning until the onset of the experiments (4 months of age) in clear Plexiglas® cages measuring 60 × 40 × 20 cm. Adult Tyron Maze Dull S3 rats (originally derived from Cpb, TNO, Zeist, The Netherlands), known as reliable aggressors (27), were used as resident males in the social stress test ("resident-intruder test," see below for details). Each TMD S3 rat was permanently housed in a wooden cage (85 × 60 × 50 cm) with a female (that was sterilized by ligation of the oviducts) to induce and preserve high levels of aggression toward strange male conspecific intruders (20). Wild-type and S3 rats were kept in separate rooms with controlled temperature (21 ± 2°C). The bedding of the cages consisted of wood shavings and food and water were freely available. Wild males were kept under a fixed 12-h light/12-h dark photoperiod (lights on from 0800 to 2000 h) and S3 rats were housed under a reversed light/dark cycle (light on at 2130 h, light off at 0930 h).

Preliminary Behavioral Classification

Wild-type males were housed together with a conspecific female (sterilized by ligation of the oviducts) in wooden cages on wood shavings (85 × 60 × 50 cm) for 2 weeks. During the second week, each animal (after temporary removal of the female) was used as resident in a standard resident-intruder test (5,19) toward a conspecific male intruder for 3 consecutive days. All tests lasted 10 min and latency to first attack (in s) was measured. The attack latency (ALT, average of 3 trials) was then used as an index of individual aggressive behavior.

Surgery

One week after the preliminary behavioral testing, male wild-type rats were premedicated with atropine (1 mg/kg, IP) and diazepam (5 mg/kg, IP; Diazemuls®, Kabi Pharmacía, Stockholm, Sweden) and anesthetized with Hypnorm TM® (10 mg/kg fluanisone and 0.2 mg/kg fentanyl, IP; Janssen Pharmaceutica B.V. Tilburg, The Netherlands). They were provided with a silastic heart cannula (i.d. 0.5 mm; o.d. 0.9 mm; Dow Corning, Midland, MI) through the right jugular vein, with one end reaching the entrance of the right atrium and the other one externalized on the top of the skull according to the technique described by Steffens (29). This method allows frequent blood sampling in conscious, undisturbed, and freely moving rats (33), even in conditions of overt fighting between the opponents (20).

Procedure

After surgery, the animals were housed individually (but in full acoustic, olfactory, and visual contact) in clear Plexiglas® cages (25 × 25 × 30 cm) and were allowed to recover for 10 days before the onset of experiments. During this period, they were connected several times to a blood sampling polyethylene tubing (70 cm long, i.d. 0.7 mm, o.d. 1.4 mm) to habituate them to the sampling procedure. On the day of the experiment, the polyethylene tubing was connected 90 min before the first blood sample withdrawal. Blood samples of 0.5 ml were taken for determination of plasma catecholamines (CAs) and corticosterone (CS). After each sample, the same amount of heparinized donor blood was transfused through the catheter to avoid changes in hemodynamics. Donor blood was obtained from additional unstressed rats provided with permanent heart catheters. All sampling sessions were performed between 1000 h and 1300 h. Wild rats were randomly assigned to 3 experimental categories: CONTROL (CTR, $n = 7$), SOCIAL DEFEAT TEST (SD, $n = 19$), SHOCK -PROD TEST (SP, $n = 16$). Blood samples were taken from all the experimental animals in baseline (from $t = -10$ to $t = 0$ min), test (from $t = 0$ to $t = 15$ min) and posttest (from $t = 15$ to $t = 60$ min) conditions. Baseline samples were withdrawn with the animal in its own home cage at $t = -10$ and $t = -1$ min. Subsequently, SD males were individually introduced into the home cage of an S3 aggressive rat after removal of the female (resident-intruder test), where they were vigorously attacked (attack latency always < 60 s). SP males were exposed in their own home cage to a shock prod, consisting of a Teflon prod with two uninsulated wires independently wrapped around it and connected to a 1000-volt shock source. Whenever the animal touched both wires simultaneously, an impedance was built up between the two wires and a DC shock (current intensity 2 mA) was delivered to the animal (7). The cumulated amount of time spent by each animal in burying the probe (burying behavior) was quantified via an electronic counter. CTR males were left undisturbed in their home cage. In all groups, test samples were withdrawn at $t = 1$, $t = 5$ and $t = 15$ min. To measure the posttest recovery of basal hormone levels, blood samples were also taken with the animal in its home cage at $t = 30$ and $t = 60$ min.

Chemical Determinations

Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01% EDTA as antioxidant and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 5°C for 10 min at 2600 rpm and 100 μl of the supernatant were stored at −80°C for catecholamines and 100 μl at −20°C for corticosterone measurements. Plasma corticosterone was measured by reversed-phase high-performance liquid chromatography (HPLC) (6). Determination of plasma catecholamine concentrations was performed by means of HPLC in combination with electrochemical detection (ECD), according to the technique described by Smedes and colleagues (28). A detailed description of the HPLC-ECD system used for detection of plasma CA levels is reported by Korte and colleagues (25).

Statistical Analysis

The amount of burying behavior during the SP test was expressed as a % of cumulative time over the total test duration and correlated with ALT via simple regression analysis. For each animal, the two baseline measurements of the hormones were averaged and only mean values were used for statistical analysis. The response patterns of each hormone were first evaluated using a 2-way ANOVA, with stress treatment as between-subject factor (3 levels) and sampling time as repeated measures within-subject factor (6 levels). For each time-point, the ratio NA/A was also calculated and statistically evaluated using 1-way ANOVA within each stress condition. Furthermore, hormone responses

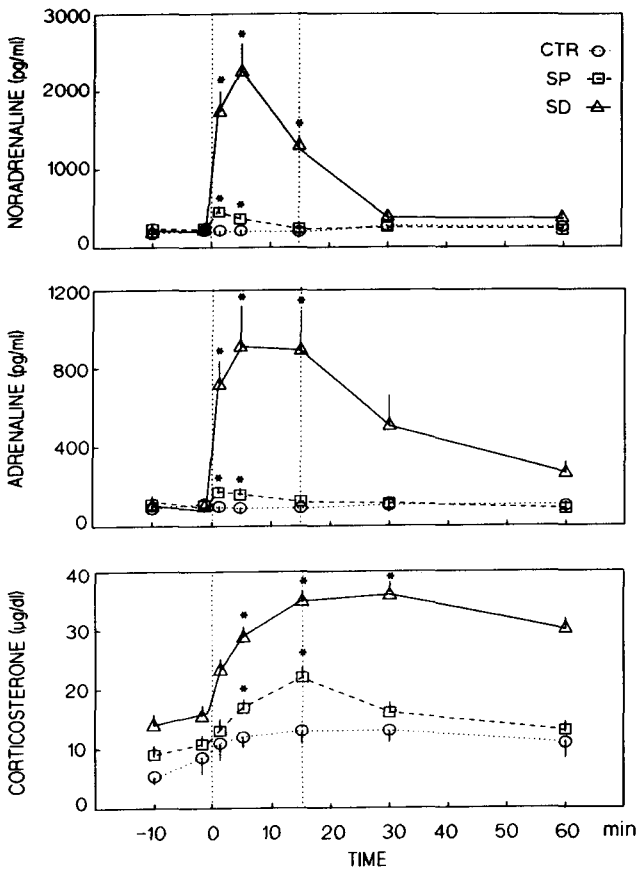

FIG. 1. Changes in plasma noradrenaline, adrenaline, and corticosterone levels in rats exposed to social defeat (SD, $n = 19$), shock-prod (SP, $n = 16$), and control (CTR, $n = 7$) test. Data are expressed as means ± SEM. ANOVA on NA, A, and CS data revealed significant Treatment (NA: F = 18.1; A: F = 8.4; CS: F = 18.7) and Time (NA: F = 9.8; A: F = 5.1; CS: F = 23.7) main effects, as well as Treatment × Time interaction (NA: F = 9.7; A: F = 5.5; CS: F = 3.5). Two-tailed Dunnett test was used after ANOVA. * significantly (at least $p < 0.05$) different from baseline values.

were quantified by computing the area under the response time curve (AUC) above the baseline. The AUC values were statistically analyzed by means of 1-way ANOVA. To determine the source of detected significance in the ANOVAs, further post hoc analyses were made by 2-tailed Dunnett and Tukey tests. A simple regression test was used to correlate the AUC values of CAs and CS in both SD and SP rats with ALT values. Where not specified, the level of significance was $p < 0.05$. Plasma levels and corresponding AUC values of the hormones were expressed as mean ± standard error of the mean (SEM).

RESULTS

Figure 1 shows the mean time-course of changes in plasma concentrations of the stress hormones before, during, and after the test in rats belonging to control (CTR), social defeat (SD), and shock-prod (SP) groups. ANOVA on NA, A, and CS values revealed significant main effects of treatment sampling time as well as a significant treatment × time interaction (see legend to Fig. 1 for detailed results of ANOVA). CTR rats showed no significant variations of plasma levels of the hormones throughout the sampling session, indicating that the blood sampling procedure did not disturb the animals.

Social defeat induced a significant increase of both NA and A as compared to baseline values at $t = 1$, $t = 5$ and $t = 15$ min, with max peaks at $t = 5$ min (2271 ± 413 pg/ml for NA and 912 ± 212 pg/ml for A). However, increased plasma adrenaline levels persisted until test termination, whereas plasma noradrenaline decreased toward baseline (NA concentrations at $t = 15$ min, though still significantly higher than baseline, were significantly lower than $t = 5$ min values) (Fig. 1). Moreover, the ratio NA/A was measured for each sample and its time evolution is reported in Fig. 2. The ratio slightly increased at $t = 1$ and $t = 5$ min compared to baseline, but decreased at $t = 15$, $t = 30$ and $t = 60$ min, suggesting that locomotor activity and associated increases in NA levels were more prominent soon after initiation of the stressor, whereas emotional distress and associated increases in adrenaline levels persisted throughout the test. CS levels were significantly raised by SD at $t = 5$, $t = 15$, $t = 30$ and $t = 60$ min, with max peak at $t = 30$ min (35.6 ± 1.9 µg/dl). Percent increment of hormone levels at the max peak as compared to pretest values were 997% for NA, 913% for A, and 130% for CS.

Also, the nonsocial stressing event of presenting a shock-prod into the home cage of the animal produced a significant increase of plasma catecholamines and corticosterone. This effect was less intense and shorter in time than SD and limited to $t = 1$ and $t = 5$ min samples for catecholamines (max peaks at $t = 1$: 456 ± 58 pg/ml for NA and 173 ± 18 pg/ml for A) and to $t = 5$ and $t = 15$ min for CS (max peak at $t = 15$: 21.6 ± 1.9 µg/dl). Percent increment of hormone levels at the max peak as compared to pretest values were 96% for NA, 60% for A, and 125% for CS. In contrast with data from SD, the ratio NA/A remained substantially unchanged throughout the sampling session in SP animals.

A quantitative comparison between the different stress situations was performed by using the area under the response time curve values (AUC) of the three hormones. Social defeat provoked a significantly higher elevation of catecholamine and corticosterone levels as compared to shock-prod presentation (ANOVA: FNA = 29.5, FA = 26.1, FCS = 24.2; $p < 0.01$, Tukey test).

Concerning the individual differentiation of the animals based on their latency to attack an intruder rat in their territory (ALT,

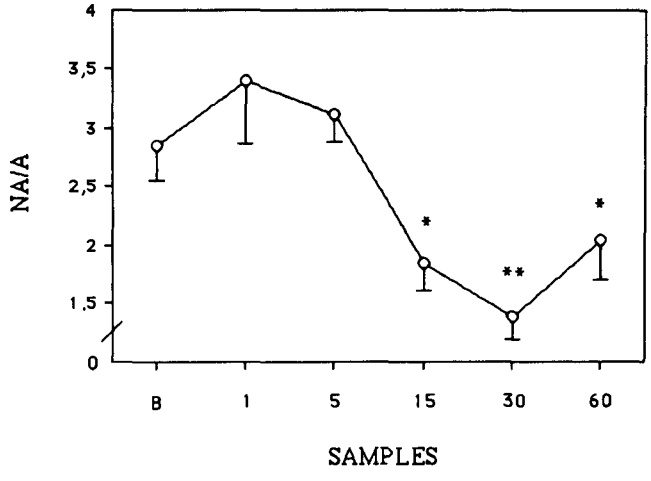

FIG. 2. Time evolution of the ratio NA/A (average values ± SEM) in animals exposed to social defeat (B = baseline, 1, 5, 15, 30, and 60 min after the beginning of the stress test). * significantly different from $t = 1$ min sample;. ** significantly different from B, $t = 1$ and $t = 5$ min samples.

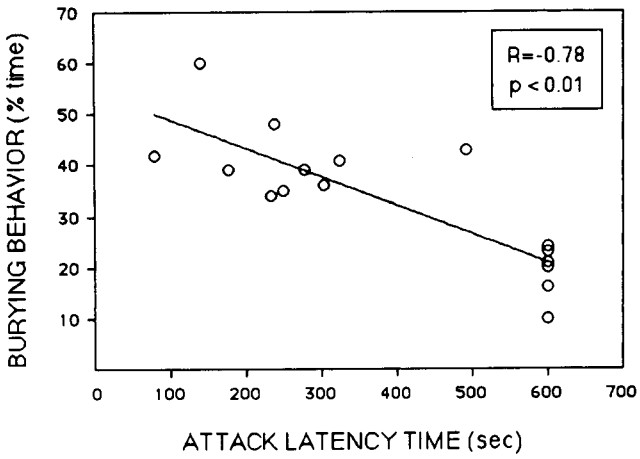

FIG. 3. Relationship (simple regression) between attack latency time (ALT, seconds) and burying behavior (% time) in rats exposed to shock-prod test (SP).

index of aggression level), it is shown in Fig. 3 that a significant negative correlation exists between the attack latency scores and the amount of burying behavior in SP rats: the more aggressive the animal (shorter latency to attack an intruder), the higher the rate of burying behavior ($R = -0.78$, $p < 0.01$). Relationships between ALT scores and baseline and AUC values of the hormones obtained in both SD and SP rats are reported in Table 1. No significant correlation between baseline concentrations of the hormones and ALT were observed. However, significant negative correlations were obtained between ALT scores and AUC values for NA and A in both SD and SP situations (i.e., the more aggressive the animal, the higher the catecholaminergic reactivity). However, no statistically significant correlation existed between ALT and the AUC for CS, neither in SD nor in the SP test. Finally, Table 2 summarizes intercorrelations among values of the AUC for NA, A, and CS. In both stress conditions, significant correlations were found only between NA and A responses.

DISCUSSION

The individual level of aggressive behavior, measured as attack latency time in the resident-intruder test, was found to be predictive of the behavior in the shock-prod burying test, as well as of the sympathetic-adrenomedullary response to this nonsocial stressing event and to the defeat stress. Aggressive males (short attack latencies) showed a more active way of responding (burying) toward an electrified shock-prod in their home cage, which was accompanied with a higher sympatho-adrenomedullary reactivity (plasma catecholamine concentrations). The significant correlation between the level of aggressive behavior as expressed by the latency to attack an intruder and the amount of burying behavior in a shock prod test is consistent with the idea that aggressive males generally adopt an active coping strategy in a challenging situation (21,23). Physiologically, this active behavioral coping style is characterized by a higher sympathetic (plasma noradrenaline) and adrenomedullary (plasma adrenaline) reactivity. The association of an active behavioral coping style with a high sympatho-adrenomedullary reactivity has been shown previously both in social (12,13) and nonsocial (7) situations.

Although it has been hypothesized that passive, low-aggressive animals react to stressful situations with a higher adrenocortical activity (17), no evidence for this association was found in this study. There appeared to be no significant correlation between the type of aggressiveness observed here and plasma corticosterone stress response.

Recent studies have questioned the generally held view of a uniform activation of the sympatho-adrenomedullary system. Sympathoneural activity, as indicated by plasma NA levels, is preferentially affected by conditions involving actual skeletal muscle exertion (physical exercise), whereas adrenomedullary stimulation, as related to plasma A levels, occurs primarily during emotional distress, fear, or anxiety-provoking situations characterized by limited or no active coping capabilities (7–9,15,32). In this study, social defeat induced much greater elevations of NA and A concentrations, indicating a higher involvement of both sympathoneural and adrenomedullary systems as compared to shock prod presentation. Moreover, the ratio NA/A indicated that the sympathoneural outflow was relatively higher in the first stages of defeat exposure (characterized by actual fighting between the opponents and flight attempts by the intruder), but decreased with the passing of time (in association with the exhibition of clear passive/submissive patterns of behavior by the intruder).

More aggressive animals actively responded to the nonsocial challenge by vigorously and frequently burying the probe: their NA levels were correspondingly higher than those measured in passively coping animals. However, this positive correlation between somatomotor activity and plasma NA concentrations was less clear for the resident-intruder test. No significant differences among individuals were observed in the amount of active behavioral responses (such as counterattack, flight, exploration) to the social

TABLE 1

RELATIONSHIPS (SIMPLE REGRESSIONS, R VALUES) BETWEEN INDIVIDUAL VALUES OF ATTACK LATENCY TIME (ALT, INDEX OF AGGRESSION LEVEL) AND BASELINE LEVELS (BAS) AND AREA UNDER THE CURVE (AUC) VALUES OF NORADRENALINE (NA), ADRENALINE (A), AND CORTICOSTERONE (CS) IN RATS EXPOSED TO SOCIAL DEFEAT AND SHOCK PROD TEST

	NA		A		CS	
	Bas	AUC	Bas	AUC	Bas	AUC
Social defeat	0.113	−0.765*	−0.066	−0.734*	0.131	−0.441
Shock prod	0.126	−0.795*	−0.102	−0.538†	0.241	0.250

* $p < 0.01$; † $p < 0.05$.

TABLE 2

RELATIONSHIPS (SIMPLE REGRESSIONS, R VALUES) AMONG INDIVIDUAL MEASUREMENTS OF THE AREA UNDER THE RESPONSE TIME CURVE (AUC) OF NORADRENALINE (NA), ADRENALINE (A), AND CORTICOSTERONE (CS) IN RATS EXPOSED TO SOCIAL DEFEAT AND SHOCK PROD TEST

	NA	A	CS	
Social defeat	1	0.734*	0.146	NA
		1	0.221	A
			1	CS
Shock prod	1	0.547†	0.075	NA
		1	0.268	A
			1	CS

* $p < 0.01$; † $p < 0.05$.

stress episode. All wild-type intruders (independently of their previous aggressive characterization) were rapidly (within the first min) and fiercely attacked by aggressive residents. Accordingly, they all exhibited typical submissive behavioral patterns (upright posture, immobility and, finally, on the back position) which covered the most time of stress exposure. From these considerations, it is quite difficult to explain the positive correlation between the level of aggression and the rate of NA increments during stress by simply claiming a different amount of physical activation.

In conclusion, these findings in an unselected strain of wild-type rats confirmed that an aggressive/active coping strategy is associated with a high sympathetic-adrenomedullary activation and support the concept of individual differentiation in coping styles as a coherent set of behavioral and neuroendocrine characteristics.

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